Development of Short Peptide Based Self-assembling Nanostructures as Drug Delivery Vehicle for Brain and as a Scaffold for Tissue Engineering

Synopsis

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INTRODUCTION

The rapidly progressing field of nanotherapeutics is being exploited to solve several limitation of conventional drug delivery system such as toxicity, lack of water solubility, poor bioavailability and low therapeutic indices (1). Various nanostructured materials have been investigated for optimal size and surface characteristics to overcome these limitations of conventional therapeutic systems. In past few years, nanostructures have also been flourished in developing methods for understanding the basis of diseases and thus devising methods to cure them (2). Various discoveries like use of gold based nanoparticles in cancer treatment through hyperthermia, application of quantum dots as biosensors and as a tool to study processes of metastasis, utilization of poly-lactic-co-glycolic acid (PLGA) based nanoparticles for drug and gene delivery, use of various polymer based hydrogels as three dimensional scaffolds for engineering of tissue in-vitro are few of the landmarks in the field of nanotechnology (3). Nanomaterials like nanoparticles on one hand improve the transport properties due to their ability to penetrate into tissue efficiently and allow a more effective delivery platform for therapeutic agents to target cells while on the other hand nanostructured materials like hydrogels provide physical support and controlled release of entrapped biomolecules for the development of tissues, intending to mimic the function of natural extracellular matrix (ECM) (4).

BACKGROUND

Research in the areas of drug delivery and tissue engineering has witnessed tremendous progress in recent years due to their unlimited potential to improve human health. Meanwhile, the development of nanotechnology provides opportunities to characterize, manipulate and organize matter systematically at the nanometer scale. Synthetic polymers such as polylactides, poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), polyethylene glycol (PEG), and poly(dl-lactide-co-glycolide) (PLGA) have been widely used for the preparation of different nanostructures (5). However, biomaterials with nano-scale organizations have also been used as controlled release reservoirs for drug delivery and artificial matrices for tissue engineering. Natural-origin polymers such as albumin, alginate, chitosan, dextran and heparin have been widely explored. Many properties of nano-scale systems can be tailored for specific applications like solubility (hydrophilicity of the material); biodistribution (molecular weight, size and shape); biocompatibility (electrical charge and other functional parameters); biodegradability (backbone); drug encapsulation

(physical interaction between drug and carrier); drug release (physical interaction with biomolecules and cells); and mechanical properties (materials and chemistry employed) (6).

Various nanomaterial based delivery systems such as gold nanoparticles, carbon nanotubes, magnetic nanoparticles, virus based nanoparticles, Polymeric micelles (polyethylene glycol), Liposomes, lipoproteins, albumin bound nanoparticles, and self-assembled peptide nanostructures are being explored in recent years (6,7).

Peptide based nanostructure:

Over the last 20 years, self-assembled nanostructures based on small peptides gained a significant interest and become biomaterials of choice. Potential applications of peptide based nanomaterials are widespread which include drug delivery, sensing agents, bioelectronics, micro-fluidic systems, tissue engineering etc. (8,9). Peptides are molecules formed by combination of amino acids linked by peptide bonds through the dehydration-condensation reaction. Peptides can be obtained conveniently from the products of proteolysis, direct synthesis by the body, or artificial synthesis (10). Peptide based nanostructures can also play an important role in the diagnosis and treatment of various diseases. Several advantages like mild synthesis conditions, fast synthesis, low-cost, relatively easy functionalization confirms the promise of peptide based nanostructures as excellent candidates for clinical use (8–11).
Peptide based nanostructures could be attained using single peptide or oligomeric polypeptides into certain morphological structure via non-covalent interactions including ionic or hydrophobic interactions, hydrogen bonding, and π-π stacking (12). Specific configurations could be obtained such as nanofibers, nanotubes nanotapes, or nanoribbons in one dimension; hydrogel scaffolds in three dimensions, if the peptide sequences are delicately modulated. The reasons that short peptides are the favourite of scientists to design nanostructures for clinical applications lie in their numerous advantages over other organic or inorganic nanostructures (11). Properties like their unmatched biocompatibility, ease of synthesis, easy surface functionality as well as tunable bioactivity along with the availability of rich chemistry for fine-tuning the structure and function of peptides according to environmental conditions make self-assembled peptide based nanostructures attractive in this field (12).

OBJECTIVES

Objective 1- Developing short peptide based self-assembling nanoparticles as carrier system for delivery of drugs to brain.

Objective 2-Developing short peptide based self-assembling hydrogels as drug delivery and tissue engineering scaffold.

WORK PLAN

Objective 1- Developing small peptide based self-assembling nanoparticles as carrier system for delivery of drugs to brain for the treatment of glioblastoma.

1) Synthesis of short peptides (dipeptides) containing α, β-dehydrophenylalanine residue (ΔF) at their C-terminal using solution phase peptide synthesis.
2) Physiochemical characterization of nanostructures formed by self-assembly of dipeptides using Dynamic Light Scattering, Electron microscope, zeta potentiometer etc.
3) To investigate whether brain endothelial cells can uptake these short peptide based nanoparticles with or without functionalization using FACS and fluorescence confocal microscopy.
4) To investigate the ability of peptide based nanostructures to cross BBB *in-vitro* using Boyden chamber or transwell assay.
5) To determine the drug entrapment efficiency.
6) To determine cellular uptake of nanoparticles with and/or without cargo into human glioblastoma cells.
7) To investigate *in-vitro* efficacy of peptide nanostructures carrying anticancer cargo for glioblastoma therapy.
8) Evaluation of cytotoxicity of peptide nanostructures *in-vitro*.

Objective 2- Developing short peptide based self-assembling hydrogels as drug delivery and tissue engineering scaffold.

1) Synthesis of small peptides using solution phase and/or solid phase peptide synthesis.
2) Physiochemical characterization of hydrogels formed by self-assembly of peptides using Dynamic Light Scattering, Electron microscopy, zeta potentiometer etc.
3) To evaluate rheological properties of peptide hydrogels for strength of stability of hydrogel structure.

4) To determine biocompatibility of peptide hydrogels in vitro using cell viability MTT [3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide] assay.
5) To evaluate potential of peptide hydrogels to support cell growth and proliferation *in-vitro* using MTT assay and confocal microscopy.
6) To investigate *in-vivo* efficacy of peptide hydrogel as three dimensional scaffold for tissue regeneration in a bone defect model.

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